excess ADP into the NMIIB environment (1 mM ADP, 1 mM ATP), which prolonged the strongly bound states further, exhibiting behavior of a molecule with high ADP affinity. We will discuss these kinetic measurements, as well as the mechanical measurements, extracted from the optical tweezers experiments in the context of NMIIB's role as a tension generating motor *in vivo*.

2552-Pos Board B522

Protein-surface Interactions and Functional Geometry of Surface-adsorbed Myosin Motor Fragments

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Biophysical studies with myosin motor fragments (heavy meromyosin; HMM and subfragment 1; S1) adsorbed to artificial surfaces, are important for elucidation of actomyosin function. In spite of the widespread use of such *in vitro* motility assays and single molecule studies, little is known about the adsorption geometry and effects of protein-surface interactions on the motor properties. Here, we investigate these factors with focus on HMM using quartz crystal microbalance with dissipation (QCM-D) and total internal reflection fluorescence (TIRF) spectroscopy based ATPase assays. In the latter, we monitored the turnover of Alexa-fluor647-ATP (Alexa-ATP) by surface adsorbed HMM. Studies were performed with HMM/S1 adsorbed to model hydrophilic (SiO_2) or hydrophobic (trimethylchlorosilane [TMCS] - derivatized) surfaces. The results suggest that adsorption of HMM is weakened on SiO_2 (but not on TMCS) at high (245 mM) compared to low (65 mM) ionic strengths. The changes in ionic strength were also associated with structural changes in the protein layer according to QCM-D studies. Moreover, the TIRF based ATPase assay suggested a larger fraction of HMM molecules with low catalytic activity on SiO_2. These and other TIRF and QCM-D results, suggest that HMM preferentially adsorbs to negatively charged hydrophilic surfaces via the actin-binding region. In contrast, the majority of the HMM molecules seem to adsorb via their C-terminal tail on moderately hydrophobic surfaces. In the latter case the catalytic sites appear to be close to, but not immobilized on the surface. The results with HMM were compared to, and found consistent with, QCM-D and TIRF-data obtained with S1 motor fragments.

2553-Pos Board B523

Simultaneous Measurement Of Actin Sliding Velocities And Actin-myosin Dissociation Kinetics

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Myosin is an enzyme that couples ATP hydrolysis with mechanical force and motion generation through its cyclic interactions with actin filaments. In order to better understand how actin-myosin mechanics and biochemistry are linked, we have developed a novel in vitro motility assay that allows us to measure actin sliding velocities, V, and actin myosin dissociation kinetics, T_{on}, simultaneously. To measure T_{on} during a motility assay, we monitor changes in actin filament dynamics near a myosin binding site using nanometer tracking of actin filaments. Actin-myosin binding damps actin dynamics, and T_{on} is estimated as the average duration of these binding events. With this approach, we observe that the duration of actin myosin binding events decreases linearly with increasing ATP concentrations, resulting in an estimated ATP induced dissociation rate constant of 0.4 μM^{-1} s^{-1}, consistent with previous kinetic measurements. This assay provides a powerful tool for simultaneous measurement of actin-myosin mechanics and the kinetics and allows us to determine the mechanochemistry of a single myosin molecule functioning within the context of an ensemble system.

2554-Pos Board B524

Actin-Myosin Binding Kinetics in Relation to Actin Sliding Velocities

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Muscle shortening velocities are widely thought to be solely limited by ATP-induced actin-myosin dissociation; however, recent studies indicate that muscle shortening velocities are influenced significantly by the kinetics and energetics of myosin's weak-to-strong binding transition. To test this hypothesis, we have developed a novel assay that allows us to measure actin-sliding velocity, *V*, and actin-myosin binding, k_{on}, and dissociation, k_{off}, kinetics in the same flow chamber. Using this assay we study the effects of different modulators of muscle mechanics such as blebbistatin, vanadate, sucrose, ATP, ADP, and P_i on k_{on}, k_{off}, and *V*. In general, when k_{off} is varied (e.g., by varying [ATP]), we observe a strong correlation between actin-myosin dissociation kinetics and *V* as predicted by detachment-limited models of *V*. However, certain modulators such as blebbistatin and vanadate affect *V* by altering weak-to-strong binding kinetics, k_{on}. This assay will allow us to better understand the mechanism by which changes in k_{on} affect *V*.

2555-Pos Board B525

Electoron Microscopy on The Comprehensive Structural Change during Actomyosin Sliding

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We have been studying the transient intermediate configuration of myosin crossbridges during in vitro sliding, utilizing a novel procedure to quantitatively compare quick-freeze replica microscopic images with the atomic models in various conformations. Snapshots of actin-sliding myosin predominantly consisted of kinked configuration to hold actin filaments inside its curvature. The images of those myosin heads cannot be reconciled with any crystal structures documented in the data-base. We also found that such unusual configuration well resembles the images of myosin molecules whose two reactive thiols are chemically-crosslinked; the putative intermediate analogue. Since myosin heads were too small for single-particle-analysis coupled with conventional electron cryo-microscopy, we attempted to determine its structure from above high-contrast replica images after classification and averaging in each class. Euler-angles of the motor-domain and the leverarm were estimated as reported, and the molecule's envelope was reconstructed from a few projections utilizing a new procedure. By including such novel configuration with different attachment angles to actin, we proposed a revised scheme on the structural change during crossbridge-cycle that comprehensively explains all the observed images under sliding conditions. Since the leverarm of the novel intermediate was kinked to the opposite direction of that in ADP/Pi-bound head, flipping must occur sometime after the head dissociates from actin filament. We thus sought for crossbridges in any other configurations in the original replica images, and found a minor fraction that resembles the ADP/Vi-form. The existence of such structure might add further information on the time-sequence of the conformational change, suggesting that once dissociated myosin in ADP/Vi-form first reattaches to actin, followed by flipping of the lerverarm to form the predominant, oppositely-kinked crossbridges. We will also show some results with a new landscape-marker probe that might be useful for the electron microscopic structural determination.

2556-Pos Board B526

A Viscous Drag Model of Frictional Loading Assays for Myosin Motors

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The myosin superfamily is a diverse family of actin binding proteins that hydrolyze ATP and generate force. The *in vitro* motility assay, which combines the strengths of solution biochemistry with the ability to measure myosin force and motility, has allowed the molecular events that couple ATP hydrolysis to mechanical work to be probed. Several groups have used modifications of the *in vitro* motility assay to study the effects of load on actin filament velocity. One such method uses alpha-actinin to exert a frictional load on sliding actin filaments that are being propelled over a bed of a given myosin isoform. This method, which has been used to measure myosin isometric force, relies on the ability to determine the amount of alpha-actinin necessary to stop actin translocation over the bed of myosin. However, careful examination of frictional loading assay data shows that even in the presence of very high concentrations of alpha-actinin, actin filament sliding was never observed to completely stop, suggesting a non-linear relationship between the amount of alpha-actinin added and the frictional load acting on the myosin. In order to better characterize this behavior, we developed a model in which the alpha-actinin behaves as a viscoelastic rather than purely elastic element, giving a velocity dependence to the frictional load imposed by the alpha-actinin. This correction allows for the determination of the myosin isometric force in the absence of a clear zero velocity point. Furthermore, this model describes the absolute resisting force imposed by the alpha-actinin as function of velocity, allowing for the measurement of isotonic kinetics using the motility assay. Supported by NIH-HL077280, AHA-0435434T (J.R.M.), and AHA-0815704D (to M.J.G.).